SENR Research Distinction

Poor Pawpaw Production in Woodland Patches- What's the Explanation?

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Table of Contents

Abstract 2

Introduction 3

Objective 4

Methods 4

Data Collection 4

Data Analysis 6

Pollination Experiment 7

Results 7

Discussion 13

Bibliography 16

Appendices 17

Appendix A 17

Appendix B 22

Abstract

The pawpaw (*Asimina triloba*) is a small fruit-bearing tree in the Annonaceae family, known for being the only tree native to the United States that bears large edible fruit (Callaway, 1990). These trees are of high cultural importance to the state of Ohio and the surrounding region for their history as a reliable and nutritious food source, an indicator of good ecological condition and, due to their clonal growth, effective erosion control. Although common in the region, fruit production is often limited, and large-scale cultivation and commercialization has not been historically successful. To determine what affects fruit production in the wild, we studied the environmental conditions of 10 different pawpaw patches in Columbus, Ohio. Five plots were located at the Olentangy River Wetland Research Park, and five at Waterman Farm, both sites managed by The Ohio State University. We assessed tree height, basal diameter, evidence of damage, and counted the number of primary branches, flowers and fruits. We also recorded woodland canopy cover and invasion by non-native woody vegetation. We monitored 377 trees in total; the mean number of flowers was 43.69 and the mean number of fruits 1.93. However both of these varied substantially at the tree level with flowering effort ranging from 0 to 274 and fruit production from 0 to 42; 265 of the 377 trees examined did not have fruit. We used a Linear-Mixed Effect Model to assess controls on tree-level flower and fruit production. Variation between plots was large but tree age, as indicated by diameter, was a tolerable predictor. Further analysis will determine the relationships between other factors, and to examine fruit production at the plot level. Our analysis provides a starting point for more research regarding how to better manage woodland pawpaw patches for fruit production. For example, understanding the influence of tree diameter on fruit production can help determine

management practices for stand structure such as spacing, thinning, or burning. This information will help continue renewed interest in effective pawpaw cultivation and also create a more reliable local food source.

Introduction

Research on pawpaws has been considerably low in the past. Although a historic food source and important fruit in cultural terms, scientific publications on the species are not as well represented in scientific literature. *A. triloba* has been previously used to study the dynamics of clonal understory species (Hosaka, 2008, Pomper, 2009), but studies designed specifically for the species have focused mainly on it's natural pesticidal value, neurotoxicity, and other phytochemical properties rather than fruit production (Lewis, 1993, Levine, 2015, Slater, 2014).

Local foods have recently increased in popularity, and because pawpaw trees are so common, pawpaw fruits could prove successful as a nutritious food source to those who are interested (Giovannucci, 2010). However, many pawpaw trees are seen to produce no fruit over several years, hindering their popularity. Determining what affects their fruiting success specifically could improve the pawpaw's popularity in the wild or in artificial landscapes, either way promoting native plants and sustainable food sources in general. Because pawpaws are ecologically beneficial in many environments, boosting their popularity would benefit not only foragers or pawpaw producers, but also ecosystems entirely (Callaway, 1990, Maxwell, 2016, Slater, 2014).

Figure 1: From left to right – pawpaw trees, flowers, young developing fruit following pollination, and mature pawpaw fruit collected by the authors from experimental woodlots in Columbus, OH.

Objective

Our objectives were to 1) determine why many large amounts of pawpaw trees produce no fruit, 2) determine what environmental variables affect fruiting and flowering effort, and 3) to determine if pollination was playing a role in fruiting success. These objectives will be met by observing ten pawpaw patches over the course of their reproductive season, identifying variables that relate to fruiting and flowering effort most, and observing differences between pollen sources and their subsequent success in producing fruit.

Methods

Data Collection

10 circular plots were selected based on structure and canopy type to ensure variation in the data collection. Each plot had a 20 meter diameter, which was measured with Keson

tape. Five plots were at the Waterman research forest and five were at the Olentangy Wetlands Research Park, both locations owned by Ohio State. These locations can be seen in **Figure 2**. The Olentangy Wetlands Research Park is a riparian area that often floods, experiences frequent foot traffic, and is heavily invaded by Amur honeysuckle (*Lonicera maackii*). Waterman is a large woodlot with little to no foot traffic, open canopy areas due to the loss of *Fraxinus spp.* from Emerald Ash Borer, and is less invaded than the wetlands, although *L. maackii* is found in various plots. At each location we recorded each tree's height, diameter, number of flowers, number of fruits, number of primary branches, and whether or not it was damaged. Damage included broken branches, callused bark, or a split top. Each plot's amount of canopy cover was also recorded, along with the presence of *L. maackii*. Flower amounts were recorded in May, while fruit amounts were recorded in late August through September. Height was recorded with a clinometer, but many of the plots were too invaded to see the desired tree from a chain away, so the raw data on the clinometer was recorded and then converted to actual heights at a later time. Also, flower amounts were only recorded for the 10 trees nearest to their plot center due to particularly dry weather that was causing flowers to prematurely fall from branches. Values for these trees were recorded specifically as those for only "count trees".

Figure 2: Site locations in Columbus relative to OSU's north section of campus

Data Analysis

Data was combined and then analyzed at the plot level using R software. Height and diameter were analyzed as the average value per plot, while fruit counts, flower counts, primary branch counts, and basal area were analyzed as sums. The presence of damage, invasion, and trees that bore fruit were analyzed as proportions. All of this data, along with canopy cover, was then incorporated into a correlation analysis to see how compatible each variable was with one another. A factor analysis was then run to combine variables into related factors. These factors were combined into Linear-Mixed Effect Models, and the best model was chosen for log-transformed fruiting and flowering data according to the AIC score. Throughout this, site was used a random effect. Factor analyses were specifically chosen to observe the inter-relationships among environmental variables, so significance was not of concern to us, thus eliminating the need for p-values.

Pollination Experiment

A small pollination experiment was conducted alongside the main study, to see if pollination was affecting fruiting or flowering effort at the wetlands location. Pollen was collected from count trees by hand, and then selected at random to pollinate other trees. Three trees were randomly chosen at each plot to be hand pollinated with pollen from a tree at the same plot (SPDT), a tree at the same site but a different plot (SSDP), and a tree from Waterman (DS).

Results

377 trees were observed in total. The mean number of flowers was 43.69 and the mean number of fruits was 1.93. These variables varied greatly, as flowering effort ranged from 0 to 274 and fruit production from 0 to 42. 265 of the 377 trees that were examined did not have any fruit on them.

Figures:

Table 1 (below): Results of the factor analyses for flower production. Orange indicates positive relationships while blue indicates negative. Lighter colors indicate a weak relationship (>0.3), while darker colors indicate a strong relation (>0.9). Factor names were renamed according to their correlations rather than the names given by R (Factor 1, Factor 2, etc.). This factor analysis was used to identify four specific factors that were influencing flower production. Factor One was renamed the “Invasion Factor” as it was highly related to the presence of *L. maackii* and the amount of canopy cover per plot. This factor accounted for 34.9% of the variance. Factor Two was renamed the "Density Factor" because it was highly related to trees per plot and the total basal area of that plot, and it explained 21.5% of the variance. Factor Three, or the "Age Factor" was related to the average diameter per plot and explained 18.3% of the variance. Factor four was retitled the "Damage Factor" as the amount of damage per plot was most related to it, and this factor accounted for 8.1% of the variance. The cumulative variance was 82.8%.

	Invasion Factor	Density Factor	Age Factor	Damage Factor
Canopy Cover	0.842	0.131	-0.158	0.168
Stem Number		0.777	-0.481	-0.392
Damage Amount	0.181			0.547
Invasion	0.977		0.140	0.142
Average Height	-0.482	0.261	0.377	0.339
Average Diameter			0.992	
Total Basal Area		0.974	0.207	
Primary Branches	-0.921	0.274	0.139	

Table 2 (below): Results of the factor analyses for fruit production. Refer to **Table 1** for the meaning of colorations. Here, Factor One was retitled "Density Factor", because like with flowering effort, stem number and basal area were involved. This factor explained 31.3% of the total variance. Factor Two, or the "Invasion Factor" was again, most related to invasion and canopy cover, and explained 22.9% of the variance. Factor Three, or the "Age Factor" was most related to average diameter per plot and it explained 21.1% of the variance. And lastly, instead of a damage factor, Factor Four was retitled "Flowering Factor" because of its relation to flowering effort. This factor accounted for 9.0% of the total variance. The cumulative variance was 84.4%

	Density Factor	Invasion Factor	Age Factor	Flowering Factor
Canopy Cover		0.994		
Stem Number	0.995			
Damage Amount	-0.329			-0.291
Invasion	-0.224	0.812	0.167	-0.432
Average Height	-0.376	0.207	0.400	-0.162
Average Diameter			0.990	
Total Basal Area	0.637		0.742	-0.126
Primary Branches	0.948	-0.276	0.122	
Total Flowers		-0.144	-0.115	0.979

Table 3 (below): results of the mixed-effect model, displaying the factor influences over flowering effort. These were combined as such to achieve the best possible AIC score in our Linear-Mixed Effect Models. The Invasion Factor had the largest effect, followed by Density, Age, Damage, and Density and Age combined.

	Estimate	Standard Error	t-value
Invasion Factor	-182.076	122.286	-1.489
Density Factor	-102.436	147.023	-0.697
Age Factor	-5.394	125.143	-0.043
Damage Factor	-38.715	121.074	-0.320
Density*Age Factor	10.098	174.506	0.058

Table 4 (below): Results of the mixed-effect model, displaying the factor influences over fruiting effort. These were combined as such to achieve the best possible AIC score in our Linear-Mixed Effect Models. Notice that the Flowering Factor was not included. Again, the Invasion Factor had the largest effect, followed by Density and then Age.

	Estimate	Standard Error	t-value
Density Factor	0.381	0.123	3.100
Invasion Factor	0.460	0.123	3.740
Age Factor	0.200	0.123	1.630

Figure 3 (below): Plot of the predicted values of flowers per plot, versus the actual values of flowers per plot, with R^2 = 0.249, found from our Linear-Mixed Effect Model.

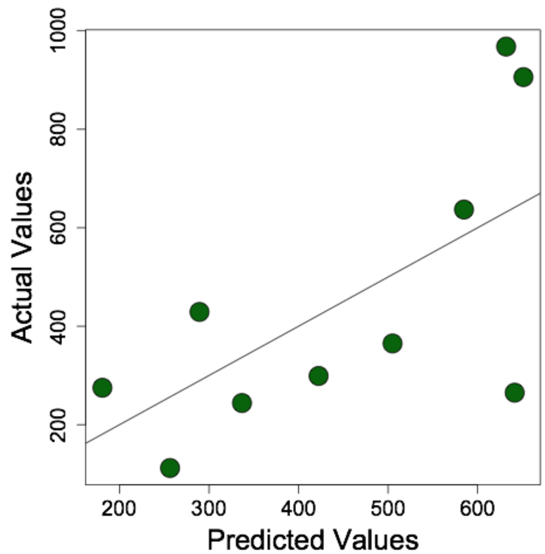


Figure 4 (below): Plot of the predicted values of fruits per plot, versus the actual values of fruits per plot, with R^2 = 0.775, found from our Linear-Mixed Effect Model.

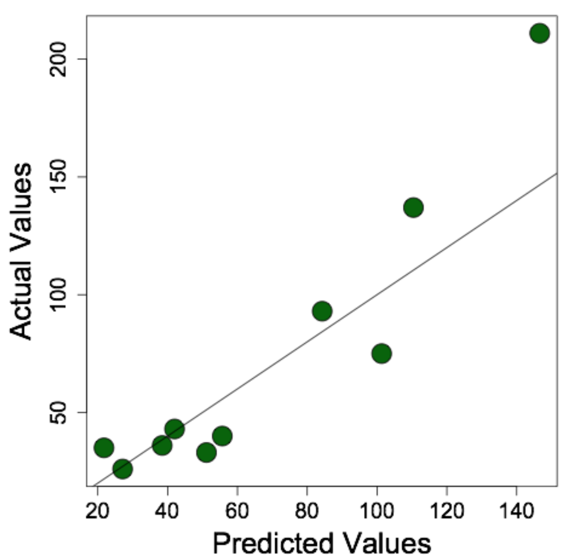


Figure 5 (below): Flowers per tree on plots 1-10. Olentangy plots are dark green, while Waterman plots are light green. This style of plot was chosen to highlight any sort of outliers that may exist on certain plots, such as the one observed in plot WA.3 and OR.4 (Waterman 3 and Olentangy 4, respectively).

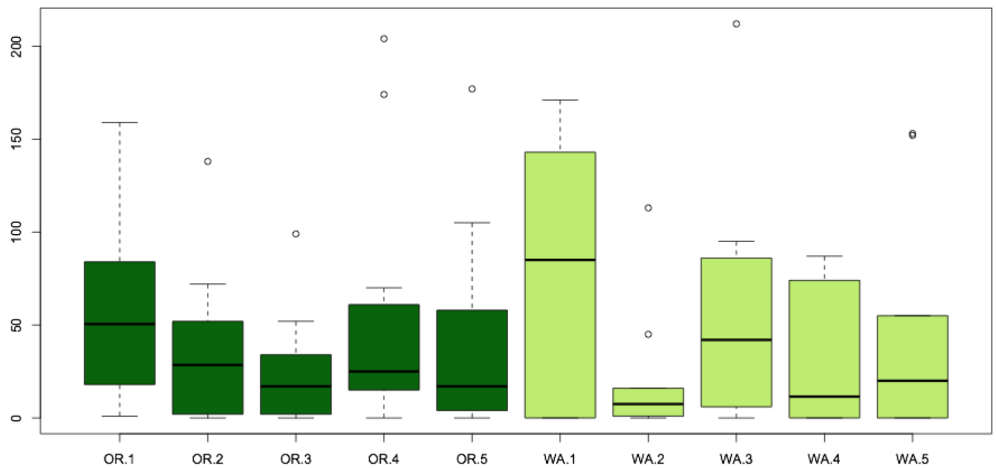
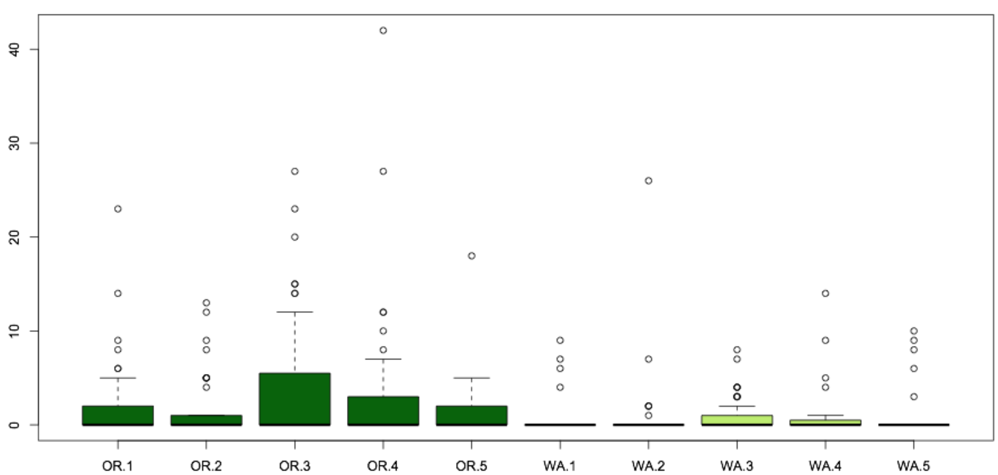


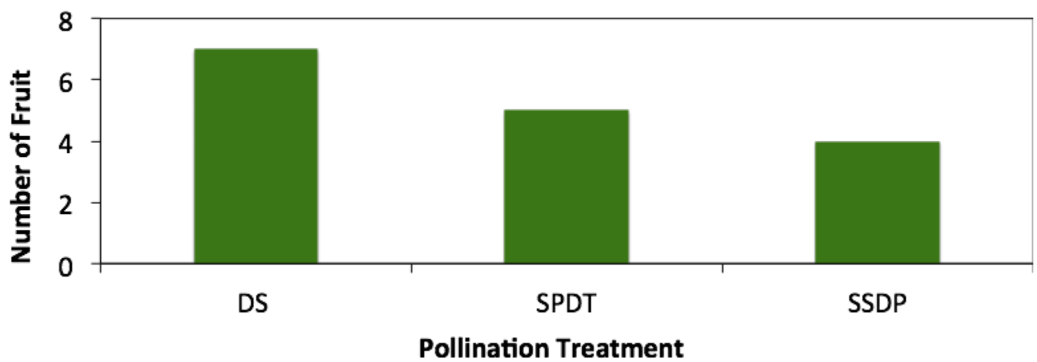
Figure 6 (above): Fruits per tree on plots 1-10. See **Figure 5** for coloration meanings. Again, plot OR.4 (Olentangy 4) displayed outliers.



Pollination Experiment

Figure 7 (Below): The results of small pollination experiment that was conducted at the Olentangy wetlands park.

- Pollen from a tree at the same plot = SPDT
- Pollen from a tree at the same site but a different plot = SSDP
- Pollen from a tree from Waterman = DS



Discussion

Several general trends were noticed from the results. For one, larger trees tended to produce more fruit and flowers, which indicated a relationship between age and fruiting/flowering production. For future research, more research regarding age and productivity is necessary. Older/larger trees seemed to produce more fruit, as stated, but how tree size correlates to age can be variable, and whether this was an isolated instance is still unknown.

Another interesting trend we found was that flowering efforts did not affect fruiting efforts. This may be in part to the large amount of flowers, but relatively small amount of fruit, thus skewing our statistics. In all of our plots the amount of flowers was disproportionately high compared to the number of fruits. This disparity may indicate an issue regarding pollination, but more research is necessary to confirm this. This key limitation should be removed if possible in future research, as it made statistical analysis difficult, and observing this occurrence in other pawpaw patches may prove helpful.

Also, stocking density had a positive effect on fruiting and flowering production, contradicting our expectation that it would cause environmental intraspecific competition, hence less overall production. We are unsure of why this is the case, but understanding this is useful to pawpaw producers when planning their stocking densities in nurseries and/or orchards.

Additionally, in many plots large total fruit production was controlled by a small number of especially productive trees, as seen in **Figure 6**. This leads us to believe that genetic factors may be playing a role in fruit counts. More research regarding genetic factors could prove useful, especially when considering these certain outliers. We did not isolate a reason for their high productivity, so understanding why these certain trees were so productive could prove crucial to improving pawpaw producer's growing stock.

The results of the flowering and fruiting Factor Analyses (**Table 1** and **Table 2**) showed that Invasion, Canopy Cover, and Primary Branches were all highly related to each other. We assumed that a high amount of *L. maackii* would positively affect canopy cover, which would thus crowd *A. triloba* trees. This may cause them to grow upwards, rather than outwards, reducing their number of primary branches. A lack of branches may then inhibit flowering effort. For fruiting effort, a small amount of branches would also hinder fruiting space, and thick amounts of honeysuckle could also prohibit pollinators from access to flowers.

Tables 1 and **2** also showed that stem number, basal area and primary branches are all related to one another. As mentioned earlier, these positive relationships contradicted our expectations that high densities of pawpaws would crowd and hinder outward growth, lowering the total basal area. We also expected high densities of pawpaws to lower primary branch totals due to crowding. However, this was not the case, as Amur honeysuckle was the main cause of vertical growth rather than intraspecific crowding.

As seen in **Table 3** and **Table 4,** the results of the mixed-effect models showed that the invasion factor, which was composed of the invasion, canopy cover, and branching variables, again had the most influence over fruiting and flowering effort. Density was the next most influential factor, followed by age. Specifically, flowering effort was also influenced by the amount of damage while fruiting effort was not.

When considering the influence of invasion, we saw that studies have shown that pawpaw trees can be particularly aggressive, but not to the extent of *L. maackii*. These two species are commonly seen competing for understory space, and *L. maackii* often is more successful (Loeb, 2010). Understanding these relationships better would not only prove useful to pawpaw producers, but in future eradication and prevention of honeysuckle invasion.

Phytochemical components of Amur honeysuckle could also be affecting pawpaw production. Many studies have shown that *L. maackii* creates allelopathic effects that can chemically suppress other potential species growth (McEwan, 2012, Wilson, 2013). Although Amur honeysuckle was not completely eliminating pawpaw stems from our patches, soil analysis is suggested to better understand the chemical effects of its presence.

The pollination experiment aligned with our expectations that pollen from the farthest away source was the most successful at producing fruit. We expected this because pawpaws are clonal, so patches of them are often related, making it difficult for trees in a patch to pollinate one another. However, this was a small experiment, so no conclusions can be reasonably made. Scientific research on *A. triloba* pollinators is relatively lacking, so no other possible explanations for an absence of successful pollination is confirmed yet. Observing this process on a larger basis could help better understand how issues facing many woodland patches, such as habitat fragmentation, pollution, and exploitation, are affecting pollinators.

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Appendices

Appendix A

Script from R software for all data analysis:

```
### PAWPAW ANALYSIS - PLOT LEVEL ###

pawpaw <-read.table(file.choose(),header=T) #data.actual.final.txt

rownames(pawpaw) <- pawpaw$plot

pawpaw$invaded <- ifelse(pawpaw$invaded=="y",1,0)
```

```
#TOTAL NUMBER OF FLOWERS
#1 Data distributions -> histogram dependent variables (appropriate model
family/transformation)
#2 Correlation between predictors
#3a Choose uncorrelated predictors to use (examine scattergraphs, include flowers in
correlation)
#3b Examine PCA to create uncorrelated predictors
#4 GLMM

#1 Data distribution
hist(pawpaw$t.flowers)
hist(pawpaw$t.fruit)

#2 Correlation analysis
pawpaw.cor <- cor(pawpaw[1,3:ncol(pawpaw)])

#3a Choose uncorrelated predictors (NB still some weak correlations in
       #variables chosen below
#Based on correlation of predictors we can compare the following models:
#Cover + Diameter
#Cover + Height
#Invasion + Diameter
#Invasion + Height
#Branching

#3b PCA to create uncorrelated predictors

#Create data frame of predictors
flower.pred <- as.data.frame(pawpaw[,3:6])
flower.pred$height.c <- pawpaw$height.c
flower.pred$diam.c <- pawpaw$diam.c
```

```
flower.pred$basal.area <- pawpaw$basal.area

flower.pred$branch.c <- pawpaw$branch.c


pca.flwr <- princomp(flower.pred)

fact.flwr <- factanal(flower.pred, factors=4, scores="regression")

flwr.facts <- as.data.frame(fact.flwr$scores)


#Factor analysis provides 4 nice uncorrelated predictors relating to:

#1 Shading effect (cover + invasion)

#2 Average tree size (height, diameter, [branching])

#3 Competition (tree number, basal area)

#4 Damage


#4 GLMM

library(lme4)


#Fit mixed model for cover + diameter

flwr.lmm1 <- lmer(pawpaw$t.flowers ~ pawpaw$cover + pawpaw$diam.c +

(1|pawpaw$location))

summary(flwr.lmm1)


#replacing diameter with height.c

flwr.lmm2 <- lmer(pawpaw$t.flowers ~ pawpaw$cover + pawpaw$height.c +

(1|pawpaw$location))

summary(flwr.lmm2)



#Fit model based on patch structural "factors"

flwr.lmm.f <- lmer(pawpaw$t.flowers ~ flwr.facts$Factor1 + flwr.facts$Factor2

* flwr.facts$Factor3 + flwr.facts$Factor4 + (1|pawpaw$location))
```

```
summary(flwr.lmm.f)

AIC(flwr.lmm.f)

#AIC = 86.58

#Residuals look ok - checked using plot(flwr.lmm.f)

#Checked fitted versus actual values using plot(predict(flwr.lmm.f),pawpaw$t.flowers)


#Factor analysis provides 4 nice uncorrelated predictors relating to:

#1 Shading effect (cover + invasion) and reduced branching

#2 Competition (tree number, basal area)

#3 Average tree size (height, [diameter] but also a bit to patch density)

#4 Flowering effort [+branching - invasion]


#4 GLMM for count tree fruit production


fruit.lmm.f <- lmer(log(pawpaw$t.fruit.c) ~ fruit.facts$Factor1 + flwr.facts$Factor2
+ flwr.facts$Factor3 + flwr.facts$Factor4 + (1|pawpaw$location))


#4 Best uncorrelated "normal" predictors for all tree fruit production


#Fit mixed model for cover + diameter

fruit.lmm4 <- lmer(pawpaw$t.fruit ~ pawpaw$cover + pawpaw$diam.all +
(1|pawpaw$location))

summary(fruit.lmm4)


#for all fruit

pawpaw.cor <- cor(pawpaw[1,3:ncol(pawpaw)])

summary (pawpaw.cor)

fruit.pred <- as.data.frame(pawpaw[,3:6])

fruit.pred$height<- pawpaw$height.a;;

fruit.pred$diameter <- pawpaw$diam.all
```

```
fruit.pred$basal.area <- pawpaw$basal.area
fruit.pred$branch <- pawpaw$branch.all

pca.fruit <- princomp(fruit.pred)
fact.fruit <- factanal(fruit.pred, factors=4, scores="regression")
fruit.facts <- as.data.frame(fact.fruit$scores)

#4 GLMM
library(lme4)

#for count fruit
pawpaw.cor <- cor(pawpaw[1,3:ncol(pawpaw)])
summary (pawpaw.cor)
fruit.c.pred <- as.data.frame(pawpaw[,3:6])
fruit.c.pred$height<- pawpaw$height.c
fruit.c.pred$diameter <- pawpaw$diam.c
fruit.c.pred$branch <- pawpaw$branch.c

pca.fruit.c <- princomp(fruit.c.pred)
fact.fruit.c <- factanal(fruit.c.pred, factors=4, scores="regression")
fruit.c.facts <- as.data.frame(fact.fruit.c$scores)

#4 GLMM
library(lme4)
```

Appendix B

Picture of bags made to protect hand-pollinated flowers: